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· 基础研究 ·

白藜芦醇通过抑制MAPKs/NF-κB信号通路治疗小鼠种植体周围炎

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【摘要】　目的　研究白藜芦醇（resveratrol，RSV）对丝线结扎诱导的实验性小鼠种植体周围炎（peri-implantitis，PI）的保护作用及其作用机制。**方法**　本研究已通过单位伦理委员会审查批准。拔除40只C57BL/6小鼠右侧上颌磨牙待自然愈合8周后在第一磨牙位点植入种植体；随机将小鼠分为对照组、小鼠种植体周围炎模型组、20 mg/kg白藜芦醇低剂量组（RSV-L）和40 mg/kg白藜芦醇高剂量组（RSV-H），植入种植体4周后，除对照组外其它小鼠建立丝线结扎诱导的种植体周围炎模型，其中模型组予以生理盐水灌胃干预，药物组用白藜芦醇灌胃干预，连续6周。观察种植体周围牙龈的水肿情况，显微CT测量小鼠种植体周围骨吸收情况；酶联免疫吸附试验（enzyme linked immunosorbent assay，ELISA）检测龈沟液中肿瘤坏死因子-α（tumor necrosis factor α，TNF-α）和白细胞介素-6（interleukin-6，IL-6）的含量；HE染色观察小鼠种植体周围组织炎性细胞浸润情况；蛋白印迹法（Western blot，WB）检测牙龈组织中细胞外调节蛋白激酶（extracellular regulated protein kinases，ERK）、p-ERK、c-Jun氨基末端激酶（c-Jun N-terminal kinase，JNK）、p-JNK、p38丝裂原活化蛋白激酶（p38 mitogen activated protein kinase，p38 MAPK）、p-p38MAPK、核因子κB（nuclear factor kappa-B，NF-κB）、p-NF-κB、核因子-κB抑制蛋白（nuclear factor-κB inhibitory protein，IκBα）、p-IκBα等蛋白表达水平及蛋白磷酸化情况。**结果**　对照组、白藜芦醇低剂量组和高剂量组治疗效果与模型组相比，组织水肿减轻，牙槽骨吸收减少，其中白藜芦醇高剂量组与低剂量组相比，组织水肿更轻，骨吸收更少；显微CT结果显示，模型组小鼠在近中、远中、颊侧和腭侧向4个位点均可观察到种植体周围骨水平发生显著的改变，高剂量白藜芦醇干预后可以减少牙槽骨的吸收（$P<0.05$）；与低剂量相比，高剂量组骨吸收在腭侧吸收减少（$P<0.05$），在近中、远中和颊侧吸收差异不显著（$P>0.05$）；ELISA结果显示，与模型组比较，白藜芦醇低剂量组、白藜芦醇高剂量组小鼠龈沟液中TNF-α、IL-6的水平较低（$P<0.05$），白藜芦醇高剂量组小鼠龈沟液中IL-6低于低剂量组（$P<0.05$），但TNF-α含量两组差异不显著；HE染色显示白藜芦醇治疗后小鼠炎性细胞浸润减少；WB结果显示，与对照组比较，模型组小鼠牙龈组织的p-Erk、p-JNK、p-p38MAPK、p-IκBα和p-NF-κB磷酸化蛋白表达水平显著升高（$P<0.01$），白藜芦醇处理组显著抑制p-Erk、p-JNK、p-p38MAPK、p-IκBα和p-NF-κB等蛋白的磷酸化，高剂量组与低剂量组相比，抑制MAPKs/NF-κB信号通路相关蛋白的磷酸化更显著（$P<0.05$）。**结论**　白藜芦醇可缓解丝线结扎诱导的实验性小鼠种植体周围炎，其机制可能是通过抑制MAPKs/NF-κB信号通路相关蛋白磷酸化。

【关键词】　种植体周围炎；　白藜芦醇；　动物模型；　p38丝裂原活化蛋白激酶；　核因子κB
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Resveratrol treats peri-implantitis in mice via inhibiting the MAPKs/NF-κB signaling pathway　LIU Senqing[1], ZHANG Hua[1], CHEN Yanyan[1], HE Haipeng[1], HUANG Jiamin[1], YUAN Jingyi[2], HU Tianyong[1], DU Ruitian[1]. 1. De-

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【Abstract】 Objective To investigate the effect of resveratrol (RSV) in the treatment of peri-implantitis in a murine model and its effect on nuclear factor kappa-B (NF-κB) signaling and mitogen-activated protein kinase (MAPKs) signaling. **Methods** This study has been reviewed and approved by the Ethics. After extracting the right maxillary molars of 40 C57BL/6 mice and allowing them to heal naturally for 8 weeks, implants were implanted at the site of the first molar. The mice were randomly divided into a control group, a mouse peri implantitis model group, a low-dose group of 20 mg/kg resveratrol (RSV-L), and a high-dose group of 40 mg/kg resveratrol (RSV-H). After 4 weeks of implant implantation, a silk thread ligation induced peri implantitis model was established in all mice except for the control group. The model group received intervention with physiological saline by gavage, while the drug group received intervention with resveratrol by gavage for 6 consecutive weeks. After 6-week treatment, observe the swelling of the gums around the implant and measure the bone resorption around the mouse implant using micro CT. Enzyme linked immunosorbent assay (ELISA) was used to detect the levels of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) in gingival crevicular fluid. HE staining was used to observe the infiltration of inflammatory cells in the surrounding tissues of mouse implants. Protein expression level and phosphorylation level of extracellular regulated protein kinases (ERK), p-ERK, c-Jun N-terminal kinase (JNK), p-JNK, p38 mitogen activated protein kinase (p38 MAPK), p-p38MAPK, nuclear factor kappa-B (NF-κB), p-NF-κB, nuclear factor-κB inhibitory protein (IκBα), p-IκBα in MAPKs/NF-κB signaling pathway were detected by Western blot (WB). **Results** Resveratrol group showed reduced tissue edema and decreased alveolar bone resorption. Among them, the high-dose resveratrol group had lighter tissue edema and weaker bone resorption compared to the low-dose group. The micro CT results showed that significant changes in the bone level around the implant were observed in the model group mice at four sites: proximal, distal, buccal, and palatal. High dose resveratrol intervention reduced alveolar bone resorption ($P < 0.05$); compared with the low-dose group, the high-dose group showed a decrease in palatal bone resorption ($P < 0.05$), while there was no significant difference in absorption between the mesial, distal, and buccal sides ($P > 0.05$). The ELISA results showed that compared with the model group, the levels of TNF - α and IL-6 in the gingival crevicular fluid of mice in the low-dose and high-dose resveratrol groups were lower ($P < 0.05$). The IL-6 in the gingival crevicular fluid of mice in the high-dose resveratrol group was lower than that in the low-dose group ($P < 0.05$). However, there was no significant difference in TNF-α levels between the two groups. HE staining showed a decrease in inflammatory cell infiltration in mice after treatment with resveratrol. The WB results showed that compared with the control group, the expression levels of p-Erk, p-JNK, p-p38MAPK, p-IκA, and p-NF-κB phosphorylated proteins in the gingival tissue of the model group mice were significantly increased ($P < 0.01$). The resveratrol treatment group significantly inhibited the phosphorylation of p-Erk, p-JNK, p-p38MAPK, p-IκA, and p-NF-κB proteins. Compared with the low-dose group, the high-dose group inhibited the phosphorylation of MAPKs/NF-κB signaling pathway related proteins more significantly ($P < 0.05$). **Conclusion** Resveratrol protect ligature induced peri-implantitis murine model, which may be related to its inhibition of phosphorylation of MAPKs/NF-κB pathway.

【Key words】 peri-implantitis; resveratrol; animal model; p38 mitogen activated protein kinase; nuclear factor kappa-B
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种植体周围炎是细菌引起的功能性种植体周围组织的炎症，并造成种植体周围骨组织破坏丧失的疾病，特点为种植体周围黏膜炎症及渐进性的支持骨组织丧失[1-4]。种植体周围炎使牙槽骨丧失并可能导致种植体脱落，是种植修复失败的主要原因之一，文献报道约10%～20%的种植体植入后会发生种植体周围炎[5-7]。种植体周围炎严重影响种植修复的长期效果，如何更有效地防治种植

体周围炎一直是口腔种植学领域的难题。

近年来，许多天然小分子药物被应用于口腔相关疾病的研究中，其中芒果苷[8]、小檗碱[9]和车叶草苷[10]等小分子药物在种植体周围炎的治疗中表现出积极的作用。白藜芦醇也是一种天然多酚类小分子药物，已被证实具有抗氧化[11]、抗炎[12-14]、抗菌[15]和调节免疫[16]等多种药理活性作用。本研究将探讨白藜芦醇对丝线结扎诱导实验性种植体周围炎小鼠的保护作用及其机制，以期为白藜芦醇用于种植体周围炎临床防治提供科学依据。

1　材料和方法

1.1　主要仪器和试剂

种植体（威高，中国），丝线（金环，中国），高分辨率活体显微CT（micro-computed tomgraphy，micro-CT）（Skyscan 1176，布鲁克，比利时），酶标仪（SpectraMax Paradigm，美谷分子，美国），全能型成像系统（ChemiDoc MP，伯乐，美国），基础电源（Power PaceBasic，伯乐，美国），滤纸条（Whatman，英国），白藜芦醇（R107315，阿拉丁），蛋白抗体兔抗鼠β-actin（4967s，CST，美国），兔抗鼠细胞外调节蛋白激酶（extracellular regulated protein kinases，ERK）（9102s，CST，美国），兔抗鼠p-ERK（9101s，CST，美国），兔抗鼠p38丝裂原活化蛋白激酶（p38 mitogen activated protein kinase，p38 MAPK）（9212s，CST，美国），兔抗鼠p-p38MAPK（9211 s，CST，美国），兔抗鼠c-Jun氨基末端激酶（c-Jun N-terminal kinase，JNK）（9252s，CST，美国），兔抗鼠p-JNK（9251s，CST，美国），鼠抗鼠核因子-κB抑制蛋白（nuclear factor-κB inhibitory protein，IκBα）（4814S，CST，美国），鼠抗鼠p-IκBα（9246S，CST，美国），兔抗鼠核因子κB（nuclear factor kappa-B，NF-κB）（8242s，CST，美国）和兔抗鼠p-NF-κB（3033 s，CST，美国），羊抗兔IgG（12-348，Sigma-Aldrich，美国）；羊抗鼠IgG（12-349，Sigma-Aldrich，美国），）和白细胞介素-6（interleukin-6，IL-6）Elisa试剂盒（CSB-E04639m-IS，武汉华美，中国）和肿瘤坏死因子-α（tumor necrosis factor α，TNF-α）Elisa试剂盒（CSB-E04741m-IS，武汉华美，中国）。

1.2　实验动物

雄性c57BL/6小鼠购于广东省医学实验动物中心，分笼饲养，室温（25 ± 2）℃，自然光照，允许自由进食和饮水，本研究已通过深圳市耳鼻咽喉研究所实验动物伦理委员会审批（批准号：龙耳医动伦[2020]第0103号），相关实验在深圳市耳鼻咽喉研究所开展。

1.3　实验分组、造模和干预

使用牙科小镊子拔除40只小鼠的右侧上颌第一、第二和第三磨牙，拔牙位点自然愈合8周。愈合后于原右侧上颌第一磨牙位点牙槽嵴顶做近远中向切口，使用牙龈分离器翻全厚瓣暴露牙槽骨面，20# ~ 50#手动K锉逐级预备窝洞，预备过程注意轻压旋转K锉并反复使用生理盐水冲洗，最后使用特制一字形螺丝刀植入种植体。种植体骨内部分直径0.65 mm，长度1 mm，表面经酸蚀处理。种植体植入4周后获得骨结合。将40只小鼠随机平均分为对照组、模型组、RSV-L（20 mg/kg）组和RSV-H（40 mg/kg）组，将5-0丝线结扎于除对照组外所有小鼠的种植体颈部以诱导种植体周围炎，对照组小鼠无需结扎。建模后，模型组予生理盐水干预，药物组予白藜芦醇干预，连续6周，每天一次，灌胃。详细造模流程、药物处理以及种植体形态见图1。

1.4　龈沟液采集

将Whatman滤纸裁剪为0.8 mm × 4.0 mm的滤纸条，灭菌后烘干，于生物安全柜分装于EP管（4根/管），编号后电子天平称重备用（精确到0.01 mg，记m_0）。龈沟液采集时（处死当天），将小鼠全身麻醉，将滤纸条插入种植体颊侧的龈沟内，避免血液和唾液的污染，30 s后取出放回EP管，间隔1 min后，再用新的滤纸条重复上述操作，多次采集龈沟液置于EP管中，最后称重（记m_1），m_1-m_0即为采集到的龈沟液重量，计算重量后按1 kg/L换算体积，最后将样本管于-80 ℃保存。

1.5　Micro-CT分析种植体周围骨吸收量

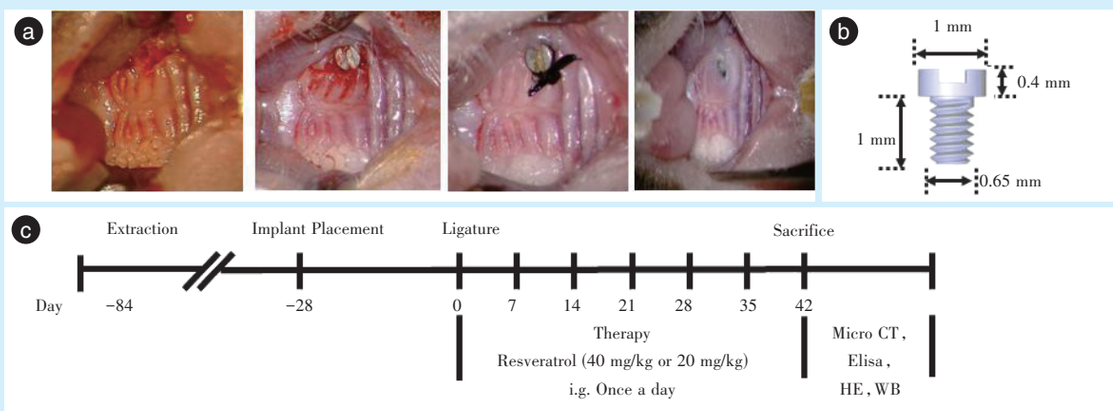
用白藜芦醇治疗42 d后，处死小鼠，每组随机抽选5个上颌骨标本浸泡于4%（*v/v*）多聚甲醛以备Micro-CT检查。扫描参数如下：工作电压80 kV，电流313 μA，图像像素9 μm。采用NReconServer软件进行三维重建，采用DataViewer软件进行测量。种植体周围骨吸收量的测量由种植体头部与体部的交点到近中、远中、颊侧和腭侧牙槽骨附着于种植体的最冠方处。

1.6　ELISA检测龈沟液炎症因子水平

检测前将收集的龈沟液滤纸从-80 ℃低温冰箱取出，加入PBS振荡（12 000 r/ min, 10 min），取上清用于ELISA检测。按照IL-6和TNF-α试剂盒说明书对各组样本中IL-6、TNF-α的含量进行检测。

1.7　HE染色观察炎症细胞浸润情况

将右上颌骨（含牙龈组织）用4%多聚甲醛进



a: clinical observations of the implant site in the development of the peri-implantitis murine model; b: representative clinical image of the implant fixtures utilized in the development of the peri-implantitis murine model; c: schematic diagram depicting timing of the experimental design; i.g.: oral gavage; HE: hematoxylin-eosin staining; WB: western blot; Elisa: enzyme linked immunosorbent assay; micro-CT: micro-computerized tomography

Figure 1　Schematic portrayal of the comprehensive experiment in mice administered with resveratrol for silk ligation-induced peri-implantitis

图1　白藜芦醇治疗丝线结扎诱导种植体周围炎小鼠实验流程示意图

行固定，EDTA脱钙，石蜡包埋，5 μm切片，乙醇梯度脱水，进行苏木红和伊红染色，光学显微镜下分析炎症细胞浸润情况。

1.8　Western blot检测MAPKs/NF-κB信号通路蛋白表达

收集各组小鼠牙龈组织，用含有磷酸化蛋白酶抑制剂的裂解液提取蛋白，采用BCA法测蛋白质浓度；经电泳、转膜等操作，5%(*v*/*v*)BSA封闭2 h后TBST洗膜(4次)，加入稀释的一抗工作液：β-actin(1∶1 000)、Erk(1∶1 000)、p-Erk(1∶1 000)、p38 MAPK(1∶1 000)、p-p38MAPK(1∶1 000)、JNK(1∶1 000)、p-JNK(1∶1 000)、IKβα(1∶1 000)、p-IκBα(1∶1 000)、NF-κB p65(1∶1 000)和p-NF-κB(1∶1 000)，4 ℃摇床孵育过夜，TBST洗膜(4次)，加入二抗工作液(1∶5 000)，放置于摇床室温孵育3 h，TBST洗膜(4次)，ECL凝胶成像分析系统扫描图像，以β-actin作为内参，最后用Image J分析和比较各组不同条带的灰度值。

1.9　统计学分析

采用Graphpad Prism 7.0进行数据统计和作图。两组间比较使用*t*检验，多组间比较使用单因素方差分析，$P<0.05$为差异具有统计学意义。

2　结　果

2.1　白藜芦醇治疗种植体周围炎小鼠的效果观察

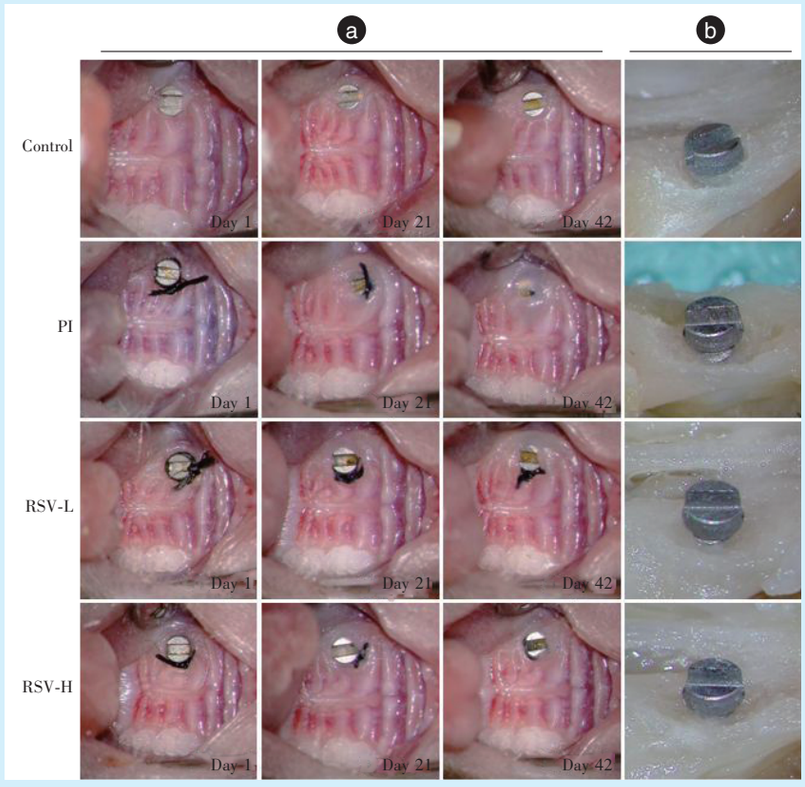
干预6周后，对照组小鼠种植体周围牙龈无炎症，模型组小鼠种植体周围牙龈明显水肿，探诊易出血，表明造模成功。白藜芦醇低剂量组和白藜芦醇高剂量组种植体周围牙龈组织上述炎症表现较轻微，临床指标优于模型组(图2a)，高剂量组炎症表现较低剂量处理组弱，水肿不明显，临床指标与正常组接近。处死小鼠后去除牙龈组织，模型组牙槽骨可见明显“碟状”吸收，治疗组相较于模型组吸收不明显(图2b)，高剂量治疗组牙槽骨吸收少于低剂量处理组。

2.2　白藜芦醇对种植体周围炎小鼠种植体周围骨水平的影响

Micro-CT影像结果显示，种植体边缘骨水平在干预6周后，模型组小鼠在近中、远中、颊侧和腭侧向4个位点均可观察到种植体周围骨水平发生显著的改变，高剂量白藜芦醇干预后可以减少牙槽骨的吸收($P<0.05$)；与低剂量相比高剂量组骨吸收在腭侧吸收减少($P<0.05$)，在近中、远中和颊侧吸收差异不显著($P>0.05$)(图3)。

2.3　白藜芦醇对种植体周围炎小鼠龈沟液中炎症因子水平的影响

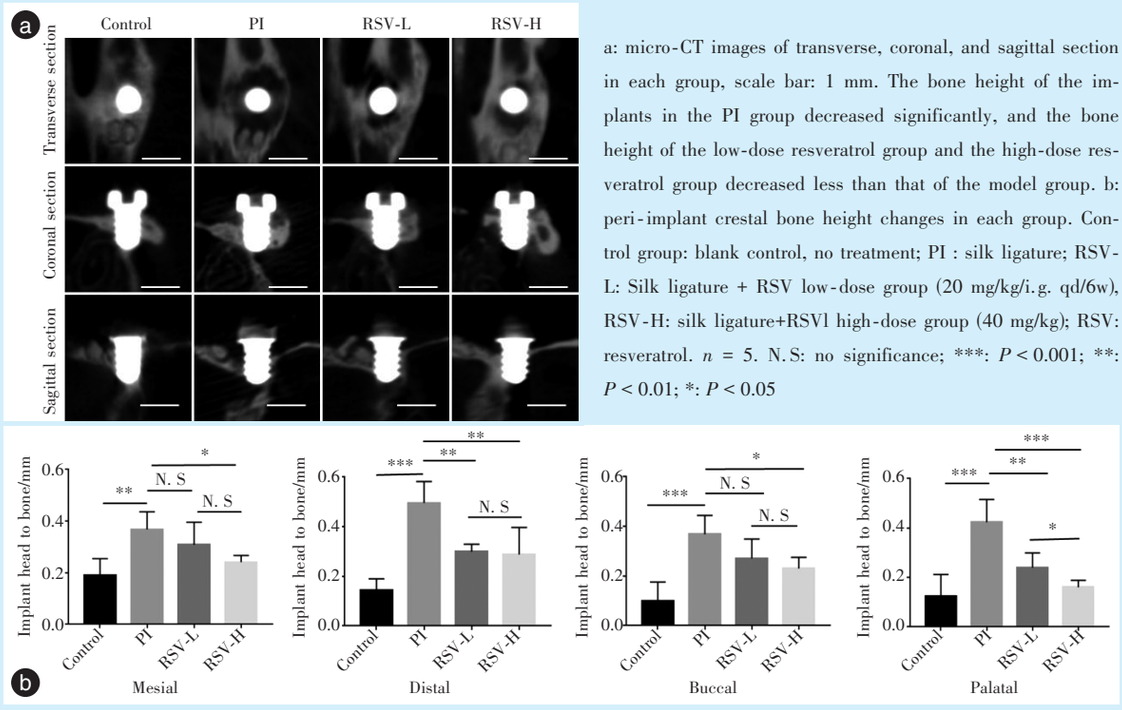
与对照组比较，模型组小鼠龈沟液中TNF-α、IL-6水平较高($P<0.05$)；与模型组比较，白藜芦醇低剂量组、白藜芦醇高剂量组小鼠龈沟液中TNF-α、IL-6的水平较低($P<0.05$)，白藜芦醇高剂量组小鼠龈沟液中IL-6低于低剂量组($P<0.05$)，但TNF-α含量两组差异不显著。见图4。



a: clinical observations of the implant site at different time points. In the PI group, there was obvious edema of the mucosa at Day 21 and 42, while the edema was less severe in the RSV-L and RSV-H groups, and there was no mucosal edema in the control group; b: alveolar bone resorption was observed. The mice were treated with resveratrol and sacrificed after 42 days, the alveolar bones were fixed with paraformaldehyde, and the tissues were carefully removed after 24 hours. The PI group exhibited "disc-shaped" resorption, while the RSV-L and RSV-H groups showed no obvious resorption, and the control group had no bone resorption. Control group: blank control, no treatment; PI : silk ligature, RSV-L: Silk ligature + RSV low-dose group (20 mg/kg/i. g. qd/6w), RSV-H : silk ligature+RSVl high-dose group (40 mg/kg); RSV: resveratrol

Figure 2　The inflammation around the implant was observed treated by RSV

图2　白藜芦醇治疗种植体周围炎的情况观察



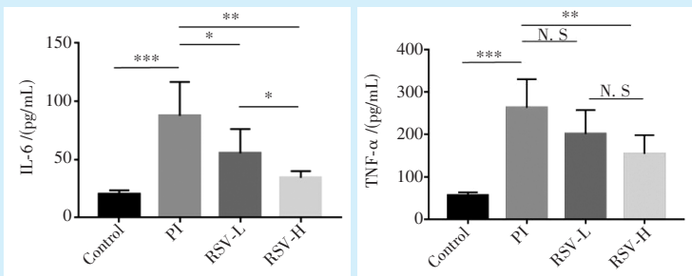
a: micro-CT images of transverse, coronal, and sagittal section in each group, scale bar: 1 mm. The bone height of the implants in the PI group decreased significantly, and the bone height of the low-dose resveratrol group and the high-dose resveratrol group decreased less than that of the model group. b: peri-implant crestal bone height changes in each group. Control group: blank control, no treatment; PI : silk ligature; RSV-L: Silk ligature + RSV low-dose group (20 mg/kg/i.g. qd/6w), RSV-H: silk ligature+RSVl high-dose group (40 mg/kg); RSV: resveratrol. n = 5. N.S: no significance; ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$

Figure 3　Changes in bone resorption levels after treatment of peri implantitis in mice with resveratrol

图3　白藜芦醇治疗小鼠种植体周围炎后骨吸收水平的变化

2.4　白藜芦醇对种植体周围炎小鼠牙龈组织形态的影响

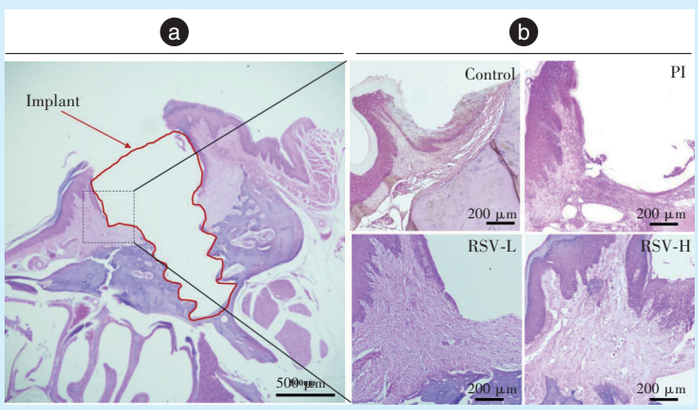
HE染色结果显示，对照组小鼠牙龈无炎性细胞浸润，模型组炎性细胞浸润较严重，白藜芦醇高剂量组和白藜芦醇低剂量组小鼠种植体周围炎性细胞浸润状况减少（图5）。



Control group: blank control, no treatment; PI: silk ligature; RSV-L: Silk ligature + RSV low-dose group (20 mg/kg/i.g. qd/6w), RSV-H : silk ligature+RSVl high-dose group (40 mg/kg); RSV: resveratrol. n = 6; N.S: no significance; ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$

Figure 4　Changes in IL-6 and TNF-α levels in gingival crevicular fluid in mice treated with resveratrol for peri implantitis

图4　白藜芦醇治疗小鼠种植体周围炎后龈沟液IL-6和TNF-α变化



Gingival tissues were stained with HE staining after 6-week treatment, In the PI group, there was obvious infiltration of inflammatory cells in the gingival tissue around the implant, while the RSV-L and RSV-H groups had less infiltration, There was no significant difference between RSV-L and RSV-H groups, and the control group had no inflammatory cells, The results indicate that RSV caused a reduction in the inflammatory tissues. Control group: blank control, no treatment; PI: silk ligature; RSV-L: Silk ligature + RSV low-dose group (20 mg/kg/i.g. qd/6w), RSV-H : silk ligature+RSVl high-dose group (40 mg/kg); RSV: resveratrol

Figure 5　HE staining of gingival tissue slices around implants in mice treated with resveratrol for peri implantitis

图5　白藜芦醇治疗小鼠种植体周围炎后种植体周牙龈组织切片HE染色

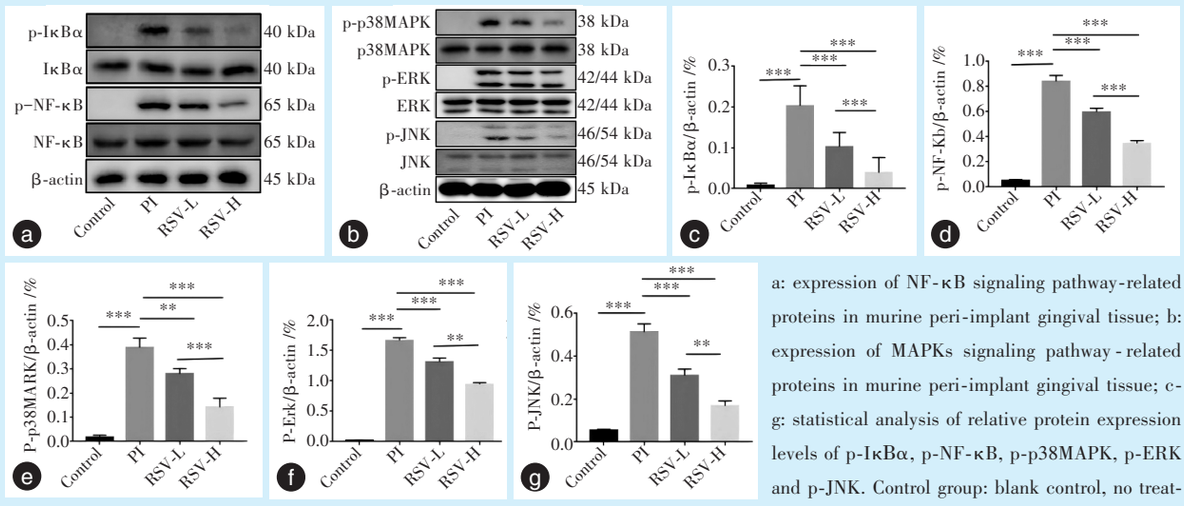
2.5　白藜芦醇对种植体周围炎小鼠牙龈组织MAPKs/NF-κB信号通路蛋白磷酸化的影响

与对照组比较，模型组小鼠牙龈组织的p-Erk、p-JNK、p-p38MAPK、p-IκBα和p-NF-κB磷酸化蛋白表达水平显著升高（$P < 0.01$），白藜芦醇处理组显著抑制p-Erk、p-JNK、p-p38MAPK、p-IκBα和p-NF-κB等蛋白的磷酸化，高剂量组与低剂量组相比，抑制MAPKs/NF-κB信号通路相关蛋白的磷酸化更显著（$P < 0.05$），表明白藜芦醇可抑制MAPK/NF-κB信号通路治疗种植体周围炎（图6）。

3　讨　论

种植体周围炎是种植修复后的常见并发症，目前认为菌斑堆积和局部炎症反应是种植体周围炎最重要的致病因素[17-18]。种植体周围炎发生后，如不及时干预会导致局部炎症信号通路的激活，加快炎症的发展，最终导致种植体松动脱落[19]。种植体周围骨组织的破坏，其核心是免疫系统失衡导致的持续性炎症，因此控制炎症的发展是治疗种植体周围炎最重要的环节。Tamaki等[11]和Ikeda等[20]分别报道白藜芦醇激活抗氧化信号通路的相关蛋白，缓解牙周炎大鼠的氧化应激状态，对牙周炎有一定的治疗作用；白藜芦醇在临床研究中具有保护牙周炎的作用[21]，白藜芦醇还可以保护肾组织损伤诱导的大鼠牙周炎[22-23]，具有抗根尖周炎作用[24]。

本研究结果显示白藜芦醇干预可以缓解种植体周围的牙龈组织炎症水肿，以及植体旁组织炎症细胞的浸润，表明白藜芦醇对小鼠种植体周围炎有抗炎作用。TNF-α是典型的促炎细胞因子，其合成和释放会招募其他细胞到炎症部位，通过增加骨髓中的破骨细胞前体直接促进骨吸收[25]；IL-6可以促进破骨细胞分化，加速骨吸收[26-27]，因此本研究检测了种植体龈沟液中TNF-a和IL-6的含量，同时用Micro-CT评估了种植体周围牙槽骨吸收情况，以确定白藜芦醇对小鼠种植体周围炎的治疗效果。研究结果显示，白藜芦醇高和低剂量组小鼠龈沟液TNF-α、IL-6含量低于模型组，micro-CT显示白藜芦醇高和低剂量干预后种植体周围骨吸收减少，表明白藜芦醇可抑制种植体周围组织的炎症因子释放并延缓牙槽骨的吸收。白藜芦醇诱导牙周膜干细胞成骨分化，可促进牙周炎小鼠模型的成



a: expression of NF-κB signaling pathway-related proteins in murine peri-implant gingival tissue; b: expression of MAPKs signaling pathway-related proteins in murine peri-implant gingival tissue; c-g: statistical analysis of relative protein expression levels of p-IκBα, p-NF-κB, p-p38MAPK, p-ERK and p-JNK. Control group: blank control, no treatment; PI : silk ligature; RSV-L: silk ligature + resveratrol low-dose group(20 mg/kg); RSV-H: silk ligature + resveratrol high-dose group (40 mg/kg). NF-κB: nuclear factor kappa-B; p-NF-κB: phosphorylation of nuclear factor kappa-B; IκBα: nuclear factor-κB inhibitory protein; p-IκBα: phosphorylation of nuclear factor-κB inhibitory protein; ERK: extracellular regulated protein kinases; p-ERK: phosphorylation of extracellular regulated protein kinases; JNK: c-Jun N-terminal kinase; p-JNK: phosphorylation of c-Jun N-terminal kinase; p38 MAPK: p38 mitogen activated protein kinase; p-p38MAPK: phosphorylation of mitogen activated protein kinase p38. ***: $P < 0.001$; **: $P < 0.01$; $n = 3$

Figure 6　Effect of resveratrol on the phosphorylation of MAPKs/NF-κB signaling pathway proteins in gingival tissue of peri implantitis mice

图6　白藜芦醇对种植体周围炎小鼠牙龈组织MAPKs/NF-κB信号通路蛋白磷酸化的影响

骨分化，推测白藜芦醇可以通过抑制炎症因子同时促进成骨分化，抑制种植体周围炎发生发展[28]。

菌斑堆积导致产生大量的脂多糖（lipopolysaccharide，LPS），LPS可激活TLR4，进而激活MAPKs和NF-κB等经典的炎症信号通路，MAPKs和NF-κB的激活最终调节炎性细胞因子的释放与表达[29]。Wu等[30]报道抑制TLR4/NF-κB可预防种植体周围炎，Jung等[31]报道抑制NF-κB对种植体周围炎有保护作用，因此在本研究检测了种植体周围牙龈组织中p-p38MAPK、p-ERK、p-JNK、P-IκBα和p-NF-κB的含量，结果显示白藜芦醇干预后牙龈组织中蛋白磷酸化减少，表明白藜芦醇可能通过下调NF-κB和MAPKs信号通路中蛋白的磷酸化治疗种植体周围炎。Tong等[28]在体外炎症模型中发现白藜芦醇可通过调控NF-κB/MAPKs信号通路抑制LPS诱导的小鼠巨噬细胞RAW264.7的炎症反应。

综上所述，白藜芦醇可下调MAPKs和NF-κB信号通路，从而调节促炎因子和抗炎因子的释放与表达，从而发挥其治疗种植体周围炎的作用。

【Author contributions】 Liu SQ performed the experiments and wrote the article. Zhang H, Chen YY, He HP and Huang JM performed the experiments and and reviewed the article. Yuan JY and Hu TY performed the experiments. Du RT designed the study and reviewed the article. All authors read and approved the final manuscript as submitted.

参考文献

[1] 周雯, 彭显, 程磊. 口腔种植体表面影响细菌黏附因素的研究进展[J]. 口腔疾病防治, 2020, 28(2): 102-106. doi: 10.12016/j.issn.2096-1456.2020.02.008.
Zhou W, Peng X, Cheng L. Research progress on factors affecting bacterial adhesion on the oral implant surface[J]. J Prev Treat Stomatol Dis, 2020, 28(2): 102-106. doi: 10.12016/j.issn.2096-1456.2020.02.008.

[2] Liu X, Deng S, Li X, et al. A standardized rat model to study peri-implantitis of transmucosal osseointegrated implants[J]. Biomater Res, 2024, 28: 0021. doi: 10.34133/bmr.0021.

[3] Herrera D, Berglundh T, Schwarz F, et al. Prevention and treatment of peri-implant diseases-The EFP S3 level clinical practice guideline[J]. J Clin Periodontol, 2023, 50(Suppl 26): 4-76. doi: 10.1111/jcpe.13823.

[4] Sahrmann P, Kühl S, Dagassan-Berndt D, et al. Radiographic assessment of the peri-implant site[J]. Periodontol 2000. 2024. doi: 10.1111/prd.12577.

[5] Derks J, Tomasi C. Peri-implant health and disease. A systematic review of current epidemiology[J]. J Clin Periodontol, 2015, 42 (Suppl 16): S158-S171. doi: 10.1111/jcpe.12334.

[6] Kordbacheh Changi K, Finkelstein J, Papapanou PN. Peri-implantitis prevalence, incidence rate, and risk factors: a study of electronic health records at a U.S. dental school[J]. Clin Oral Implants Res, 2019, 30(4): 306-314. doi: 10.1111/clr.13416.

[7] Stacchi C, Troiano G, Rapani A, et al. Factors influencing the prevalence of peri-implantitis in implants inserted in augmented

maxillary sinuses: a multicenter cross-sectional study[J]. J Periodontol, 2021, 92(8): 1117-1125. doi: 10.1002/JPER.20-0483.

[8] Li H, Chen Z, Zhong X, et al. Mangiferin alleviates experimental peri-implantitis via suppressing interleukin-6 production and Toll-like receptor 2 signaling pathway[J]. J Orthop Surg Res, 2019, 14(1): 325. doi: 10.1186/s13018-019-1387-3.

[9] 许楠, 赵雁焕, 王雯. 黄连素治疗大鼠种植体周围炎的机制[J]. 西北药学杂志, 2024, 39(3): 59-64. doi: 10.3969/j.issn.1004-2407.2024.03.010.
Xu N, Zhao YH, Wang W. Mechanism of berberine in treating peri-implant inflammation in rats[J]. Northwest Pharm J, 2024, 39(3): 59-64. doi: 10.3969/j.issn.1004-2407.2024.03.010.

[10] Wang X, Chen X, Zhang Z, et al. Asperuloside prevents peri-implantitis via suppression of NF-κB and ERK1/2 on rats[J]. Pharmaceuticals(Basel), 2022, 15(8): 1027. doi: 10.3390/ph15081027.

[11] Tamaki N, Cristina Orihuela-Campos R, Inagaki Y, et al. Resveratrol improves oxidative stress and prevents the progression of periodontitis via the activation of the Sirt1/AMPK and the Nrf2/antioxidant defense pathways in a rat periodontitis model[J]. Free Radic Biol Med, 2014, 75: 222-229. doi: 10.1016/j.freeradbiomed.2014.07.034.

[12] Gu Y, Lou Y, Zhou Z, et al. Resveratrol for inflammatory bowel disease in preclinical studies: a systematic review and meta-analysis[J]. Front Pharmacol, 2024, 15: 1411566. doi: 10.3389/fphar.2024.1411566.

[13] Huang CY, Chen SH, Lin T, et al. Resveratrol attenuates advanced glycation end product-induced senescence and inflammation in human gingival fibroblasts[J]. J Dent Sci, 2024, 19(1): 580-586. doi: 10.1016/j.jds.2023.10.016.

[14] Jiang H, Ni J, Hu L, et al. Resveratrol may reduce the degree of periodontitis by regulating ERK pathway in gingival-derived MSCs [J]. Int J Mol Sci, 2023, 24(14): 11294. doi: 10.3390/ijms241411294.

[15] Vestergaard M, Ingmer H. Antibacterial and antifungal properties of resveratrol[J]. Int J Antimicrob Agents, 2019, 53(6): 716-723. doi: 10.1016/j.ijantimicag.2019.02.015.

[16] Malaguarnera L. Influence of resveratrol on the immune response [J]. Nutrients, 2019, 11(5): 946. doi: 10.3390/nu11050946.

[17] Berglundh T, Jepsen S, Stadlinger B, et al. Peri-implantitis and its prevention[J]. Clin Oral Implants Res, 2019, 30(2): 150-155. doi: 10.1111/clr.13401.

[18] Di Spirito F, Giordano F, Di Palo MP, et al. Microbiota of peri-implant healthy tissues, peri-implant mucositis, and peri-implantitis: a comprehensive review[J]. Microorganisms, 2024, 12(6): 1137. doi: 10.3390/microorganisms12061137.

[19] 李珍. 负载二十二碳六烯酸的纳米结构脂质载体对大鼠种植体周围炎的治疗作用[D]. 长春: 吉林大学, 2023.
Li Z. Therapeutic effect of nano-structured lipid carrier loaded with docosahexaenoic acid on periimplant inflammation in rats[D]. Changchun: Jilin University, 2023.

[20] Ikeda E, Tanaka D, Glogauer M, et al. Healing effects of monomer and dimer resveratrol in a mouse periodontitis model[J]. BMC Oral Health. 2022, 22(1): 460. doi: 10.1186/s12903-022-02499-2.

[21] Nikniaz S, Vaziri F, Mansouri R. Impact of resveratrol supplementation on clinical parameters and inflammatory markers in patients with chronic periodontitis: a randomized clinical trail[J]. BMC Oral Health, 2023, 23(1): 177. doi: 10.1186/s12903-023-02877-4.

[22] Ding X, Hou Y, Liu X, et al. The role of Sirt3-induced autophagy in renal structural damage caused by periodontitis in rats[J]. J Periodontal Res, 2023, 58(1): 97-108. doi: 10.1111/jre.13071.

[23] Li X, Liu XC, Ding X, et al. Resveratrol protects renal damages induced by periodontitis via preventing mitochondrial dysfunction in rats[J]. Oral Dis, 2023, 29(4): 1812-1825. doi: 10.1111/odi.14148.

[24] 陈虹君, 雷奇, 王治林, 等. 基于网络药理学和分子对接的白藜芦醇治疗口腔鳞状细胞癌的机制研究[J]. 口腔疾病防治, 2024, 32(3): 178-187. doi: 10.12016/j.issn.2096-1456.2024.03.003.
Chen HJ, Lei Qi, Wang ZL, et al. Network pharmacology and molecular docking reveal the mechanism of resveratrol in oral squamous cell carcinoma treatment[J]. J Prev Treat Stomatol Dis, 2024, 32(3): 178-187. doi: 10.12016/j.issn.2096-1456.2024.03.003..

[25] Darabi E, Kadkhoda Z, Amirzargar A. Comparison of the levels of tumor necrosis factor-α and interleukin-17 in gingival crevicular fluid of patients with peri-implantitis and a control group with healthy implants[J]. Iran J Allergy Asthma Immunol, 2013, 12(1): 75-80.

[26] Wu Q, Zhou X, Huang D, et al. IL-6 enhances osteocyte-mediated osteoclastogenesis by promoting JAK2 and RANKL activity *in vitro* [J]. Cell Physiol Biochem, 2017, 41(4): 1360-1369. doi: 10.1159/000465455.

[27] Zhang Y, He X, Wang K, et al. Irisin alleviates obesity-induced bone loss by inhibiting interleukin 6 expression via TLR4/MyD88/NF-κB axis in adipocytes[J]. J Adv Res, 2024 doi: 10.1016/j.jare.2024.04.013.

[28] Tong W, Chen X, Song X, et al. Resveratrol inhibits LPS-induced inflammation through suppressing the signaling cascades of TLR4-NF-κB/MAPKs/IRF3[J]. Exp Ther Med, 2020, 19(3): 1824-1834. doi: 10.3892/etm.2019.8396.

[29] Guo Q, Jin Y, Chen X, et al. NF-κB in biology and targeted therapy: new insights and translational implications[J]. Signal Transduct Target Ther, 2024, 9(1): 53. doi: 10.1038/s41392-024-01757-9.

[30] Wu X, Qiao S, Wang W, et al. Melatonin prevents peri-implantitis via suppression of TLR4/NF-κB[J]. Acta Biomater, 2021, 134: 325-336. doi: 10.1016/j.actbio.2021.07.017.

[31] Jung HJ, Lee W, Shin JS, et al. The effects of NF-kB inhibition with p65-TMD-Linked PTD on inflammatory responses at peri-implantitis sites[J]. Inflammation, 2021, 44(6): 2291-2301. doi: 10.1007/s10753-021-01500-4.

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